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ON THE TOPIC

Time to mend a broken heart☆

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Myocardial infarction (heart attack) results in severe damage to the heart muscle. Post-infarction, heart function is compromised as a result of cardiomyocyte death and an acute inflammatory response that is a prelude to deposition of permanent scar tissue. Long-term molecular, cellular and physiological responses to the damaged muscle result in disease progression to heart failure. The progression from ischaemic injury to heart failure highlights the limited regenerative reserve of cardiac tissue in man. This is contrasted by an extraordinary ability of the zebrafish heart to regenerate after resection of 20% of its ventricular mass, via formation of a fibrous clot and cellular blastema, in 60 days (Poss et al., 2002). Nevertheless, multipotent cardiac stem cells appear to exist in mammals and recent genetic fate mapping experiments have revealed that a non-myocyte population can repopulate the myocardium in response to cardiac stress, including infarction and pressure overload, but do not significantly contribute to the cardiomyocyte population homeostatically during normal aging (Hsieh et al., 2007). Furthermore, recent experiments suggest that mammalian hearts might be coaxed into a regenerative model, including formation of new cardiomyocytes, by a cocktail of factors given at the time of infarction (reviewed in Ballard and Edelberg, 2007).

In humans, stem cell-based therapies offer the potential to repair the damaged heart and restrict scar formation and disease progression. Promising initial results of stem cell-based therapy in animal disease models lead to the rapid transfer of this approach from the laboratory to the clinic (Srivastava and Ivey, 2006). However, such an enthusiastic leap belies the recent emergence of the methodology and, in particular, the rudimentary understanding of the biological mechanisms underlying the improvements to heart function observed. Indeed, early positive indications for the use of bone-marrow stem cell fractions or mobilisation regimes to repair myocardium have been controversial (Kovacic et al., 2005; Srivastava and Ivey, 2006) and results from large, double-blinded, controlled, cell therapy studies involving clinical outcomes have only just started to emerge (Ballard and Edelberg, 2007).

However, the ultimate cell type or therapy for cardiac repair is at this point unknown, and direct delivery of differentiated cardiomyocytes to the diseased heart (myocardial replacement therapy; MRT) may have advantages over endogenous or exogenous stem cell-mediated repair especially in the aged, in whom both systemic and local factors that would normally support stem cell survival and deployment may have long waned (Ballard and Edelberg, 2007; Rando, 2006). Recent reports using ES cell-derived cardiomyocytes in both intra-species and xeno-transplantation studies suggest that cardiomyocytes generated in this way offer an unlimited supply of suitable cells for MRT and may improve heart function post-myocardial infarction (Kehat et al., 2004; Kolossov et al., 2006; Tomescot et al., 2007).

In this issue of Stem Cell Research, van Laake and colleges present a detailed and instructive study using human ES cell (HESC)-derived cardiomyocytes injected into healthy and diseased mouse hearts (van Laake et al., 2007). Cells

☆ For ethical reasons, the Victor Chang Cardiac Research Institute does not engage in, nor does it condone, the destruction of human embryos for research or creation of human embryonic stem cells.

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injected consisted of dissociated tissue dissected from cultures in which HESCs were differentiated in the presence of an endodermal cell line with the capacity to augment cardiogenesis, and as such represent a mixed pool with an estimated cardiomyocyte (CM) content of 20–25%. The injected CMs form a small, functioning, isolated syncytium within the normal host ventricle. In the context of cell therapy, it was promising that the engrafted immature CMs gradually matured, shown by the formation of both desmosomes and gap junctions, and activation of the adult myocardial markers *MLC2v* and *Connexin-43*. Furthermore, the CMs were positively selected for survival. These data complement those found when using mouse ES cell-derived CMs, which also formed an isolated syncytium that matured over time (Kolossov et al., 2006). Similarly, HESC-derived CMs did not functionally integrate with the host myocardium but instead were shown to remain in isolated grafts separated from host CMs by extracellular matrix. Previous studies have demonstrated integration of HESC-derived CMs into host myocardium, but only in non-ischaemic conditions (see Kehat et al., 2004; Laflamme et al., 2007). The reason for this is unknown, but may reflect the different maturation state or biophysical properties of host and graft tissue, for example, in this case, the large discrepancy between mouse and human heart rates may prevent incorporation of HESC-derived CMs into the myocardium.

Next the authors investigated the potential of the HESC-derived CMs in a mouse model of ischemic heart disease. Introduction of CMs into damaged heart tissue resulted in improved cardiac performance parameters at 4 weeks post-infarction. However, at 12 weeks post-infarction no difference was observed between experimental and control mice. This finding illustrates the importance of evaluating the long-term contribution of CMs, or indeed any engrafted cells, that appear to promote cardiac function in the shorter time frame. The analysis presented by van Laake and colleagues suggests that a non-cell autonomous mechanism initially improves heart function, but as the graft becomes established this signal is downregulated or lost, diminishing the difference in contractile function between experimental and control animals. Cautious interpretation of these data is required. While it may be tempting to suggest that the greatest level of functional support in these and other cell therapy studies is provided by trophic rather that biomechanical effects, perhaps via augmentation of vasculogenesis and cardiomyocyte survival, the transient nature of the response demands closer scrutiny. We can nonetheless remain hopeful that MRT will offer much greater levels of long-term biomechanical support to the diseased heart than are evident in this study. Technical difficulties such as the low survival rate of directly injected myocytes and their poor level of integration with host myocardium have perhaps affected the outcome in the current protocol. If proper electrical integration of myocytes can be achieved, the effect may be sustained. Future experiments using mammalian models with more closely matched host and donor cells will be necessary to carefully dissect the roles of injected cells in providing trophic and biomechanical support. In the longer term, the bland infarct model in an immunocompromised host shows a very different post-infarction inflammatory response than humans or mice whose coronary vessels have been re-perfused, and this may shape the ultimate outcome of cell therapy. Models of ischaemia/re-perfusion are well established and can be examined. Despite the many unanswered questions, the great value of this manuscript by van Laake et al. is that it highlights the necessity of looking beyond short-term outcomes in interpreting results.

In another prominent study published this year (Laflamme et al., 2007), HESC-derived CMs treated with a cocktail of pro-survival factors were injected into rat hearts after ischemia-reperfusion. Survival of CMs in the graft was selected for and improved by the cocktail, remuscularising a significant proportion of myocardium, but similar to the van Laake et al. study, they did not integrate with host myocardium. Improvements to cardiac functional parameters occurred relative to control treatments, but measurements were taken only at 4 weeks. Thus, the durability of these effects remain to be determined.

While HESC-derived CMs potentially represent an inexhaustible supply of human cardiac tissue for use in MRT, the successful implementation of this approach faces several hurdles. In addition to the difficulties inherent in any cell-based therapy (e.g. delivery method, immunological compatibility) CMs need to successfully integrate, and the integration process must also be "measured", otherwise cardiac arrhythmias may arise. Further experimentation is needed to reveal the processes required for CMs engraftment to the host myocardium and to determine the contribution of transplanted CMs to long-term improvement of cardiac muscle performance. Several different stem-cell based therapies for cardiovascular disease are currently being evaluated, including the stimulation or injection of mesenchymal stem cells, bone marrow mononuclear cells, endothelial progenitor cells and skeletal myoblasts, and the attempted activation of recently discovered endogenous cardiac stem cells (Ballard and Edelberg, 2007). Thus, stem-cell research may provide a number of complementary therapeutic approaches, including MRT, for cardiovascular disease. In recent large-scale clinical trials using bone marrow derived cells (BMDCs) there was an improvement in cardiac function and a reduction in the hard clinical endpoints of reoccurrence of infarction and death (reviewed in Fuster and Sanz, 2006). Despite these encouraging findings there is no evidence that BMDCs differentiate into cardiomyocytes or elicit a long-term regenerative response (Fuster and Sanz, 2006; Schwartz, 2006). Therefore, improved understanding of both the differentiation process and the mechanisms underlying the correct integration of transplanted cells with the host tissue will help guide MRT in the clinical setting. Cardiac muscle needs to be fed, and so a combination of MRT and pro-vascular therapy may be the next challenge in the much-anticipated transition from bench to bedside.

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